

# KIRBY-BAUER DISK DIFFUSION TEST

## Aim

Preparing plates with bacterial inoculum and adding filter papers soaked in samples to assess whether the samples have a bactericidal or bacteriostatic function.

## Procedure

1. Prepare sterile Whatman filter paper with a hole punch and also antibiotic free agar plates. For better visualisation MacConkey or Endo Agar can be used. Up to 6 samples or controls can be tested on one plate.
2. Streak 50  $\mu$ l of a bacterial culture with approximately OD<sub>600</sub> 0.6 onto the plates. After streaking in one direction, rotate the plate by 90° and repeat the spreading (without applying more liquid culture) until the plate has completed a full turn. This step is essential to assure even distribution of the bacteria!
3. Do not turn the plates upside down as usual, instead let dry at RT while preparing samples.
4. When preparing samples, make sure to include proper positive and negative controls. For cell lysates, a good negative control is lysate from untransformed cells as well as sterile water. Kanamycin at 35 ug/ml (1:1000 dilution from stock) serves as a positive control.
5. Use tweezers to place filter papers onto an empty petri dish and pipet 20  $\mu$ l of sample / control onto each paper. Let soak for a bit before 'dragging' the papers out of the liquid and dabbing them onto an empty part of the dish to remove excess liquid.
6. Place filter papers onto agar, making sure to use proper sterile technique.
7. Let the plates dry as in step (3) or place them in the incubator lid facing up if drying at RT takes too long.
8. Check your plates after 12 h (ideally) and 24 h for bacterial growth and to check for halos. Take pictures of the halos and measure their radius.

## Note!

It is very important to work with sterile equipment and reagents in this case to assure that no contaminants influence bacterial growth.

## Sources

[http://www.globe-network.org/sites/default/files/sites/default/files/documents/disk-diffusion-susceptibilities-en\\_0.pdf](http://www.globe-network.org/sites/default/files/sites/default/files/documents/disk-diffusion-susceptibilities-en_0.pdf) (retrieved 25.09.2016)

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